PB81-184715

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Mar 81

U.S. Department of Commerce
National Technical Information Service
NTIS.

EPA-600/J-80-233
Journal Article

P881-184715

Reprinted from

Canadian Journal of Fisheries and Aquatic Sciences

Réimpression du

Journal canadien des sciences halieutiques et aquatiques

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Volume 37 • Number 8 • 1980

Pages 1225–1228

PRODUCT OF:
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
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Government of Canada
Fisheries and Oceans

Gouvernement du Canada
Pêches et Océans

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/J-80-233	2. Journal Article
3. RECIPIENT'S ACCESSION NO. PB81 184715	
4. TITLE AND SUBTITLE Neanthes arenaceodentata (Polychaeta: Annelida), a proposed cytogenetic model for marine genetic toxicology	5. REPORT DATE March 1981
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Gerald G. Pesch and Carol E. Pesch	8. PERFORMING ORGANIZATION REPORT NO. ERLN-J132
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Laboratory Narragansett, RI 02882	10. PROGRAM ELEMENT NO. ACHH1A
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Laboratory Narragansett, RI 02882	13. TYPE OF REPORT AND PERIOD COVERED journal article
	14. SPONSORING AGENCY CODE EPA/600/05

15. SUPPLEMENTARY NOTES
REFERENCE: Canadian Journal of Fisheries and Aquatic Sciences, Vol. 37, No. 8, pp. 1225-1228

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
polychaetes, chromosomes, sister chromatid exchange, genetic toxicology		

18. DISTRIBUTION STATEMENT Release Unlimited	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES 4
	20. SECURITY CLASS *(This page)*	22. PRICE

EPA Form 2220-1 (9-73)

Neanthes arenaceodentata (Polychaeta: Annelida), a Proposed Cytogenetic Model for Marine Genetic Toxicology

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Key words: sister chromatid exchange, chromosome, marine worm, polychaete

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Il existe dans les environnements marins pollués des toxiques génétiques qui, à long terme, peuvent être une menace pour les populations d'organismes marins. Le présent article décrit en détail l'application in vivo d'une analyse d'échange de chromatides soeurs (SCE) à un polychète marin, *Neanthes arenaceodentata*. Cette espèce réagit à la mitomycin C (MMC) à des concentrations comparables à celles déclenchant des réponses dans des systèmes de mammifères in vivo. Une dose de 5×10^{-7} mol/L MMC accélère le taux SCE depuis une fréquence de base de 0,14/chromosome jusqu'à 0,5/chromosome. Les chromosomes de *N. arenaceodentata* peuvent fournir un modèle pour l'identification et l'étude des effets de toxiques génétiques dans les environnements marins.

Received January 3, 1980
Accepted April 23, 1980

Reçu le 3 janvier 1980
Accepté le 23 avril 1980

THE presence, effects, and consequences of genetic toxicants in marine environments have received little attention. Longwell (1976) observed chromosome damage suffered by larval mackerel collected in heavily polluted New York Bight. Parry et al. (1976) used microbial systems to detect mutagenic activity in tissue extracts of mussels collected from areas with obvious industrial pollution. These observations suggest that genetic toxicants are present in polluted marine environments and that genetic impairment may represent a long-term threat to populations of marine organisms. Sister chromatid exchange (SCE) analysis is a sensitive cytogenetic technique for measuring potential genetic hazards of environmental pollutants (Latt 1974; Perry and Evans 1975; Bloom 1978; Kligerman 1979). This paper presents details of an in vivo application of SCE analysis to a marine polychaete worm, *Neanthes arenaceodentata*. This species was chosen as a model for studying cytogenetic effects of toxicants in marine environments for two reasons. First, *N. arenaceodentata* has 18 large chromosomes and is easily cultured, thus providing a continuous supply of experimental material. Second, it is a benthic species and of particular interest because sediments are often reservoirs for pollutants. Polychaetes are important food web species for commercially important fishes and therefore may biomagnify toxicants.

Materials and Methods

Neanthes arenaceodentata construct mucous tubes within sediment or on firm surfaces. Females deposit eggs within the mucous tubes, then die; males incubate the eggs, and larvae develop within the tubes. At 20°C an entire life cycle (egg to egg) is completed in 9 to 10 wk. All of the *N. arenaceodentata* larvae used in this study were obtained from laboratory cultures that originated at California State University, Long Beach. Details of culture techniques have been published by Reish (in press).

For these experiments 8- to 10-d-old larvae were removed from the tubes and were exposed in the dark to 5-

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Printed in Canada (J5913)
Imprimé au Canada (J5913)

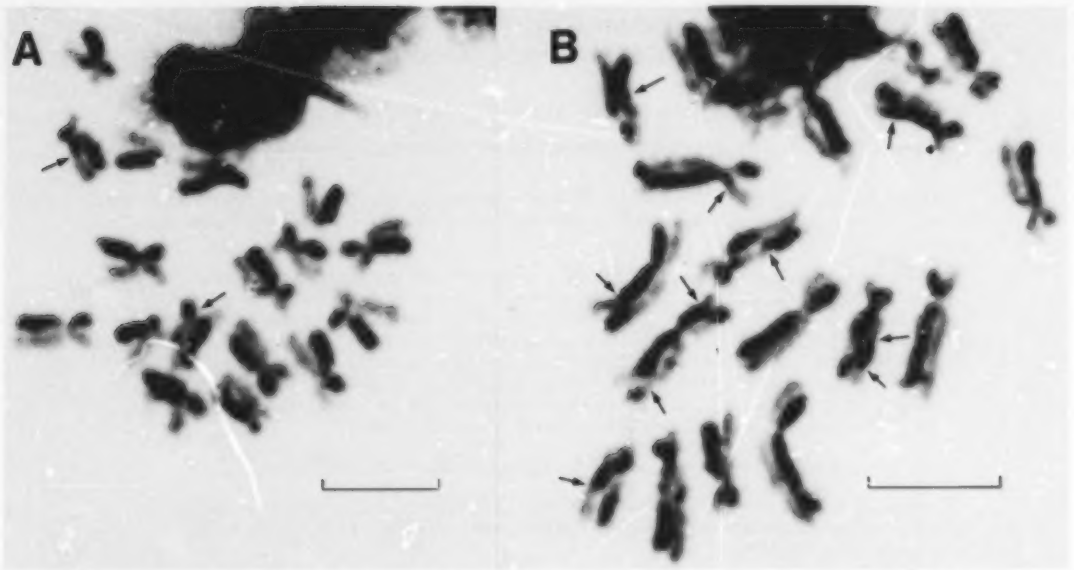

FIG. 1. Metaphase cells from the marine worm *Neanthes arenaceodentata* after two rounds of replication in the presence of BrdU (30 μmol/L) and stained with the hot-phosphate Giemsa technique. (A) Control animal, note two SCEs; (B) animal exposed to MMC (5×10^{-7} mol/L), note the increase in SCEs. Bar equals 5 μm.

bromodeoxyuridine (3×10^{-5} mol/L BrdU) in 50 mL of filtered seawater for 48 h. Mitomycin C (MMC), a known mutagen (Latt 1974), was mixed to appropriate concentrations with filtered seawater, then added concurrently with the BrdU. Colchicine (0.05%) was added to the seawater for the last 12 h of the 48-h exposure period.

The following procedure for slide preparation was adapted from Kligerman and Bloom (1977). Seawater was decanted and replaced with 100 mL of 0.075 mol/L solution of potassium chloride for 1 h, followed by larval fixation in three changes of ethanol–acetic acid (3:1) for 1 h each. Fixed larvae (25–30) were placed on a clean slide and enough 50% acetic acid was added to cover all of the larvae (several drops). After 4 min the acid was drained off and the larvae were smeared by pressing a second slide on top of the first and then drawing the two slides apart with one smooth continuous movement. This produced a monolayer of separated cells on each slide, which were air-dried for 1 wk and then stained using the hot phosphate–Giemsa technique of Korenberg and Freedlender (1974). Differentially stained metaphase spreads were scored using a Zeiss Universal microscope, 100× planapochromatic objective, 1.6× optovar, and 10× ocular. Photographs were taken with Kodak 35-mm high-contrast copy film. Data were analyzed by one-way analysis of variance and by Bartlett's test for homogeneity of variance according to procedures in Snedecor and Cochran (1967).

Results

Differentially stained chromosomes (SCD) were observed in 65 to 72% of metaphase spreads, which numbered 94 ± 30 per slide for four control slides. Because the proportion of SCD is related to exposure time to BrdU and rate of cell division, the experimental conditions used here were determined empirically and would differ for different life stages of the worm.

SCEs were scored for cells that replicated twice in the presence of BrdU and were spread well enough to be counted confidently (Fig. 1). The percentage of such cells was low; therefore, only seven were counted for each treatment. The frequency of SCE in controls was 2.5 ± 1.3/metaphase or 0.14 ± 0.07/chromosome. The range of SCE/metaphase in controls was 1 to 4.

The frequency of SCE was increased significantly ($\alpha < 0.02$) by low levels of MMC (5×10^{-8} mol/L). Higher levels of MMC further increased the number of SCEs (Fig. 1). The dose response to MMC is shown in Fig. 2.

Discussion

Latt (1974) first demonstrated the utility of BrdU-revealed SCE to screen for mutagenicity of toxicants. Perry and Evans (1975) confirmed the broad applicability of this technique. SCE is now widely used in vitro for testing chemicals. In vitro application is very useful; however, it does not simulate whole organism responses, nor does it permit exposure under complex environmental conditions. In vivo assays are needed. In vivo SCE assays have been developed for a plant species (Kihlman and Kronborg 1975), chick embryo (Bloom and Hsu 1975), two species of mam-

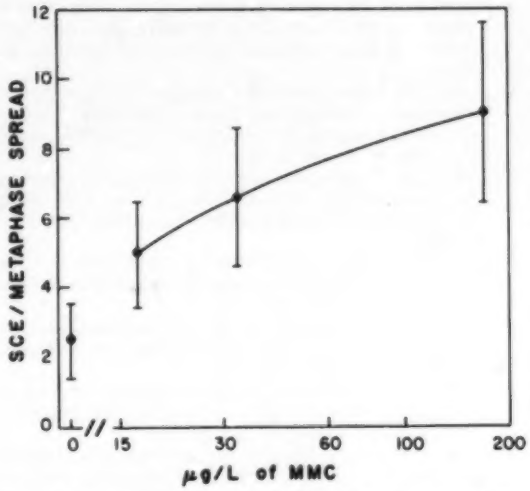

FIG. 2. Dose response curve for SCE frequencies in the marine worm *Neanthes arenaceodentata* exposed to three concentrations of mitomycin C. SCEs were scored in second division metaphase cells and each point represents a mean frequency of SCE for seven cells.

mals (Stetka and Wolff 1976; Allen and Latt 1976; Vogel and Bauknecht 1976), a freshwater fish (Kligerman and Bloom 1976), and now a marine worm.

The frequency of SCE is influenced by many factors, including the concentration of BrdU (Lambert et al. 1976). With in vivo systems there are undoubtedly differences in DNA content, exposure, distribution, and availability of BrdU within different tissues of an organism and among species. Consequently, it is not surprising that different organisms have different baseline SCE frequencies. Table 1 compares baseline SCE frequencies for a variety of in vivo test systems. With the exception of the plant, *Vicia faba*, the systems have low SCE frequencies. The two aquatic species, mudminnow and a polychaete worm, have comparable SCE frequencies despite differences in dosing and methodology. In comparisons of SCE frequencies for in vivo and in vitro systems, Kligerman and Bloom (1976) found frequencies to be higher in the in vitro systems.

Any new system for assessing genetic toxicants must be validated with known mutagens to ensure its responsiveness. Several in vivo SCE systems have been tested with MMC (Table 2). These systems responded with surprisingly similar increases in SCE to similar doses of MMC. *Neanthes arenaceodentata*, when exposed to three concentrations of MMC, exhibited a dose response to a known mutagen in an expected manner and at levels of sensitivity exhibited by mammalian systems.

Some difficulties remain with the worm assay. Although there is a high rate of SCD, there are few well-spread metaphases. Technique problems exist because of the high yolk content and refractory nature of the material. Observations on the embryology of this species have been reported by Reish (1957); however, the details needed to prescribe dosing times are missing and must be determined empirically. At present we are working with early embryos attempting to shorten dosing times and increase the number of countable metaphase spreads.

TABLE 1. Frequencies of sister chromatid exchange measured in vivo.

System	BrdU dose	SCE/chromosome	Reference
Vicia faba	100 µmol/L	1.72	Kihlman and Kronborg 1975
Rabbit	10^{-4} mol/L	0.13	Stetka and Wolff 1976
Rabbit	10^{-5} mol/L	0.14	Stetka et al. 1978
Mouse	40 µg/g	0.10	Vogel and Bauknecht 1976
Mouse	8.6 mg/mouse	0.05	Allen and Latt 1976
Mouse	1300 mg/kg	0.13	Nakanishi and Schneider 1979
Chick embryo	25 µg/embryo	0.07	Bloom and Hsu 1975
Mudminnow	500 µg/g	0.12	Kligerman and Bloom 1976
Worm	30 µmol/L	0.14	Present study

TABLE 2. Frequency of SCE response to MMC by in vivo test systems.

System	Dose of MMC	Exposed SCE/chromosome	Control SCE/chromosome	Exposed ÷ control	Reference
Mouse	0.5 mg/kg (0.5 mg/L)	0.18	0.05	3.6	Allen and Latt 1976
Mouse	1 mg/kg (1.0 mg/L)	0.5	0.13	4.0	Nakanishi and Schneider 1979
Rabbit	0.5 mg/kg (0.5 mg/L) (8× weekly)	0.5	0.14	3.6	Stetka et al. 1978
Worm	5×10^{-7} M (0.17 mg/L)	0.5	0.14	3.6	Present study

In summary, SCE can be measured in vivo in a marine worm, *N. arenaceodentata*. This species exhibited a dose response to MMC at concentrations comparable to those eliciting responses in in vivo mammalian systems. The chromosomes of *N. arenaceodentata* may provide a useful model for identifying and studying the effects of genetic toxicants in marine environments.

Acknowledgments

We thank Dr A. R. Malcolm of this laboratory for advice and encouragement; Nancy Persson and Karen Heise for secretarial assistance; Dr Henry Holden and Paula Crider of Pfizer Pharmaceutical Co., Groton, CT, and Helen Lebowitz of Litton Bionetics, Kensington, MD for their generous assistance with the fine art of SCE.

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